THE EXISTENCE OF *SPOROTHRIX SCHENCKII*[3] AS A SAPROPHYTE IN TAIWAN

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Abstract: A total of 251 native and newly naturalized plant species found in Taiwan were examined for the saprophytic growth of *Sporothrix schenckii*. The organism was isolated from soil samples throughout the country. Of the plant species tested, 114 supported no growth, 65 supported moderate growth, 37 supported good growth while 35 species induced abundant mycelial and conidial production. The studies demonstrated variation among plant species to support growth of *S. schenckii* as a saprophyte in nature.

INTRODUCTION

Soil borne keratinophilic fungi were examined in detail with particular attention to soil collections of 250 primary schools throughout Taiwan, and numerous collections from the northern, eastern and southern areas of Taiwan (Volz, *et al.*, 1974). Additional species other than those isolated from the soil have been reported in the literature as causal organisms of dermatomycoses in Taiwan (Jen, 1963, 1970). Not all dermatophytes have been isolated from soil, however the soil remains as a common reservoir.

From the several handred soil collections, *Sporothrix schenckii* Hekton & Perkins was found on 58 occasions (Volz *et al.*, 1974). The frequency of isolation brought further interest in the habitat of *S. schenckii* in Taiwan. On many occasions the literature has reported human involvement of the species associated with the growth of the organism in nature. No association of the organism in nature has been directly attributed to sporotrichosis in Taiwan, however this undoubtedly is correct. In this study an attempt is made to identify native plants associated with the saprophytic state of *S. schenckii* in Taiwan and the probability of human contact in nature.

MATERIALS AND METHODS

Dried leaves of 251 plant species collected in Taiwan (Li, *et al.*, 1975) were obtained from herbarium specimens at the National Taiwan University. Species selection included plants commonly found, thus their presence in nature significantly adds to the organic content of the soil. Each plant specimen was placed on 9.0 cm diameter number 1 Whatman filter paper, wrapped in aluminum foiland autoclaved. Sterile plant specimens and filter paper were next placed in sterile disposable Falcon plastic petri dishes. Sterile distilled water was added to each dish to saturate the filter paper and moisten the plant specimen.

Sporothrix schenckii stock cultures were maintained on Sabouraud's maltose agar slants. Ten day old colonies grown at room temperature in petri dishes were used as the inoculum. Conidial

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(3) Supported by National Science Council Grant NSC-64-0501-0202(10) to Dr. Zuei-ching Chen, Mycology Laboratory, Department of Botany, National Taiwan University.

and hyphal filaments from 1 cm square colony sections were placed on the sterile plant specimens and incubated at 26°C for two weeks. After incubation, plant species were examined for hyphal growth and conidial production of *S. schenckii* on the leaf surfaces and recorded according to hyphal growth density and abundance of conidia.

RESULTS

Growth of *S. schenckii* on selected plant species leaves was identified according to the abundance of hyphae and the degree of conidial production on leaf surfaces. Sterile distilled water and moist filter paper in petri dishes provided suitable moisture for spore germination while plant material served as the nutrient source for the fungus. Leaves were grouped into four categories according to the saprophytic growth of *Sporothrix* on the plant species.

In the first group of plants, no growth of *S. schenckii* was evident. Spore germination was minimal, no hypal development and no growth away from the inoculation source occurred. Plants found in this group included: *Achyranthes ogatai* Yamamoto, *Achyranthes rubrofusca* Wight., *Agrostis arisan-montana* Ohwi, *Aleurites moluccana* (Linn.) Willd., *Allium odorum* Linn., *Alopecurus aequalis* Sobol. var. *amurensis* (Komar.) Ohwi, *Ambrosia elatior* Linn., *Anaphalis nagasawai* Hay., *Araiostegia parvipinnula* (Hay.) Copel., *Artemisia princeps* Pampanini var. *orientalis* (Pamp.) Hara, *Arthraxon hispidus* (Thunb.) Mak., *Arundinella setosa* Trin., *Aulacolepis agrostoides* Ohwi var. *formosana* Ohwi, *Bacopa monnieria* (Linn.) Wetist., *Berchemia lineata* (Linn.) DC., *Bidens bipinnata* Linn., *Bothriospermum tenellum* Fisch. et Mey., *Brachiaria reptans* (Linn.) Gardn. & Hubb., *Brainea insignis* (Hook.) J. Sm., *Canna coccinea* Mill., *Cardiospermum halicacabum* Linn. var. *microcarpum* (H. B. K.) Bl., *Carex baccans* Nees ex Wight, *Carex brachyathera* Ohwi, *Carex sociata* Boott., *Carex subtransversa* Clarke, *Cassia leschenaultiana* DC., *Cassia mimosoides* Linn., *Cerastium echinatus* Linn., *Cerastium trigynum* Vill. var. *morrisonense* Hay., *Chrysopogon aciculatus* (Retz.) Trin., *Crotalaria albida* Heyne, *Crotalaria anagyroides* H. B. K., *Crotalaria calycina* Schr., *Cuphea cartagenensis* (Jacq.) Macb., *Cyathea podophylla* (Hook.) Copel., *Cymbopogon tortilis* (Fresl.) A. Camus., *Cyperus brevifolius* (Rottb.) Hass., *Cyperus compressus* Linn., *Desmodium heterocarpum* (Linn.) DC., *Desmodium heterophyllum* (Willd.) DC., *Desmodium sequax* Wall. var. *sinuatum* (Miq.) Hook., *Dichanthium annulatus* (Forssk.) Stapf, *Digitaria adscendens* (H. B. K.) Henr., *Dimeria orinthopoda* Trin., *Diospyros maritima* Bl., *Dracaena angustifolia* Roxb., *Drosera indica* Linn., *Dryopteris formosana* (Christ) C. Chr., *Erianthus pollinioides* Rendle, *Euonymus matsudai* Hay., *Ficus cuspidatocaudata* Hay., *Fimbristylis serices* (Poiret) R. Br., *Fimbristylis spathacea* Roth var. *umbellatocapitata* (Hill) T. Koyama, *Geophila herbacea* (Jacq.) Ktze., *Glycine soja* Sieb. & Zucc., *Gomphrena celasioides* Mart., *Goniothalamus amuyon* (Blanco) Merr., *Hedyotis tenelliflora* Bl., *Helicteres angustifolia* Linn., *Heliotropium indicum* Linn., *Hemiboea bicornuta* (Hay.) Ohwi, *Ichnanthus vicinus* (F. M. Bail.) Merr., *Ilex formosana* Maxim., *Indigofera suffruticosa* Mill., *Justicia procumbens* Linn., *Lasianthus cyanocarpus* Jack., *Lespedeza cuneata* (Du Mont de Cours.) G. Don, *Lindera communis* Hemsl., *Lindera strychnifolia* (Sieb. & Zucc.) F. Vill., *Lolium multiflorum* Lamk., *Ludwigia octovalis* (Jacq.) Raven, *Lycopodium fordii* Bak., *Lygodium microphyllum* (Cav.) R. Br., *Lysionotus warleyensis* Willm., *Mcchilus thunbergii* Sieb. & Zucc., *Marattia fraxinea* Sm., *Melochia corchorifolia* Linn., *Mesona procumbens* Hemsl., *Microstegium glaberrimum* (Honda) Koidz, *Morinda umbellata* Linn., *Mussaenda pubescens* Ait., *Myrsine sequinii* Levl., *Panicum incomtum* Trin., *Pasania randaiensis* (Hay.) Schott., *Pennisetum alopecuroides* (Linn.) Spreng., *Pharagmites karka* (Retz.) Trin., *Pipturus arborescens* (Link.) C. B. Rob., *Potentilla discolor* Bunge, *Procris laevigata* Bl., *Prunella vulgaris* Linn., *Prunus phaeosticta* (Hance) Maxim., *Pteris dispar* Kunze, *Quercus variabilis* Bl., *Rhynchosia volubilis* Lour., *Rubus rarissimus* Hay., *Rubus tagallus* Chem. et Schlech., *Salvia keitaoensis* Hay., *Scilla scilla scilloides* (Lindl.) Druce, *Scirpus ternatanus* Reinw., *Scleria rugosa* var. *glabrescens* (Koidz.) Ohwi & T. Koyama, *Scleria terrestris* (Linn.) Fassett, *Scoparia dulcis* Linn., *Sencio scandens* Roch.-Ham. var.

crataegifolius (Hay.) Kitam., *Setaria geniculata* (Lamk.) P. Beauv., *Smilax elongato-umbella* Hay., *Solanum nigrum* Linn., *Sporobolus hancei* Rend., *Stautonia keitaoensis* Hay., *Stephania cepharantha* Hay., *Trichosporum acuminatum* (Wall.) O. Kuntze, *Triumfetta bartramia* Linn., *Vandenboschia auriculata* (Bl.) Copel., *Verbena officinalis* Linn., *Vigna luteola* (Jacq.) Benth. and *Xyris pauciflora* Willd.

A second group of plants supported moderate growth of *S. schenckii*. Hyphal growth developed on the test leaf surface near the source of inoculation. Growth was not vigorous and few spores were produced. Some leaves supported hyphal growth without spore production, however, growth was independent of the inoculum, with the leaf surface providing nurients for a sparce hyphal development. Plants placed in this group included: *Actinidia callosa* Lindl. var. *formosana* Finct & Gagnep., *Actinostemma lobatum* (Maxim., *Actidesma japonicus* Sieb. & Zucc. var. *densiflorum* Hurusawa, *Arisia quinquegona* Bl., *Ardisia sieboldii* Mig., *Callicarpa japonica* Thunb. var. *kotoensis* (Hay.) Masam., *Canavalia lineata* (Thunb.) DC., *Cantharospermum scarabaoides* (Linn.) Baill., *Capillipedium parviflorum* (R. Br.) Stapf, *Christella acuminata* (Houtt.) Lev., *Cyanotis arachnoidea* Clarke, *Cyperus pilosus* Vahl., *Dendropanax trifidus* (Thunb.) Mak., *Desmodium buergeri* Mig., *Desmodium heterocarpum* (Linn.) DC. var. *buergeri* Hosokawa, *Desmodium umbellatum* (Linn.) DC., *Diospyros eriantha* Champ., *Dodonaea viscosa* (Linn.) Jacq., *Echinochloa crusgalli* (Linn.) P. Beauv., *Elephantopus scaber* Linn., *Eleusine indica* (Linn.) Gaertn., *Eurya acuminata* DC., *Ficus harlandi* Benth, *Ficus nervosa* Heyne, *Goldfussia pentemonoides* Nees, *Grewia rhombifolia* Kaneh. & Sasaki, *Gymnema affine* Decne, *Helicteres angustifolia* Linn., *Hemiboea bicornuta* (Hay.) Ohwi, *Humulus japonicus* Sieb. & Zucc., *Hyptis suaveolens* Poit., *Ilex ficoides* Hemsl., *Ilex kusanoi* Hay., *Ilex rotunda* Thunb., *Leucas mollissima* Wall. var. *chinensis* Benth., *Limnanthemum indicum* Thw., *Lonicera japonica* Thunb., *Ludwigia epilobioides* Maxim. subsp. *epilobioides* Raven, *Ludwigia perennis* Linn., *Lycopodium cernuum* Linn., *Maoutia setosa* Wedd., *Melissa parviflora* Benth., *Milletia reticulata* Benth., *Mollugo oppositifolia* Linn., *Morinda citrifolia* Linn., *Mosla dianthera* Maxim., *Ormocarpum cochinchinense* (Lour.) Merr., *Paederia scandens* (Lour.) Merr., *Paspalum conjugatum* Berg., *Perrottetia arisanensis* Hay., *Photinia beauverdiana* Schneider var. *notabilis* Rehder & Wilson, *Phytolacca acinosa* Roxb., *Pieris taiwanensis* Hay., *Platycarya strobilacea* Sieb. & Zucc., *Podocarpus polystachys* R. Br. ex Mirb., *Pogonatherum crinitum* (Thunb.) Kunth, *Scirpus subcapitatus* Thwaites var. *morrisonensis* (Hay.) Ohwi, *Selaginella mollendorffii* Hieron., *Sideroxylon ferrugineum* Hook. & Arn., *Symplocos kotoensis* Hay., *Syzygium lanyuense* Chang, *Urena lobata* Linn., *Villebrunea pedunculata* Shirai, *Wedelia chinensis* (Osbeck) Merr., and *Xanthium strumarium* Linn..

Good growth of *S. schenskii* on a third group of plants was evident. Hyphal growth was directed away from the source of inoculation and it was independent of the inoculum site. An increase in the hyphal branching system occurred with formation of a more dense hyphal mat in the hyphal growth area on the leaf surface. Conidial production of the fungus was moderate in this group of plants. The plant species included in the third group were: *Athyrium subrigescens* Hay., *Bifaria opuntia* (Thunb.) Merr., *Borreria hispida* (Linn.) K. Schum., *Celastrus punctatus* Thunb., *Corydalis koidzumiana* Ohwi, *Daphniphyllum glaucescens* Blume, *Eupatorium formosanum* Hay., *Euphorbia serrulata* Reinw., *Gardenia jasminoides* Ellis, *Gynura flava* Hay., *Juncus leschencultii* Gay, *Lasianthus chinensis* Benth., *Marattia pellucida* Presl., *Moghania strobilifera* (Linn.) J. St. Hilaire., *Monochoria vaginalis* (Burm. f.) Presl., *Mosla punctulata* (J. F. Gmel.) Nakai, *Pachycentria formosana* Hay., *Perrottetia arisanensis* Hay., *Photinia serrulata* Lindl., *Pittosporum moluccanum* Miq., *Plagiogyria glauca* (Bl.) Mett. var. *Philippinensis* Chr., *Pleocremia cumingiana* Presl., *Polygonum hypoleucum* (Nakai) Ohwi, *Polygonum orientale* Linn., *Polygonum sieboldii* Meisn., *Potamogeton octandrus* Poir., *Pseudoeurya crenatifolia* Yamamoto, *Rubus alnifoliatus* Levl., *Rubus parvifolius* Linn., *Salvia hayatana* Makino, *Scolopia oldnami* Hance, *Shortia exappendiculata* Hay., *Sida cordifolia* Linn., *Sloanea dasycarpa* (Benth.) Hemsl., *Solanum indicum* Linn., *Sorbus randaiensis* Koidz., and *Thea sinensis* Linn..

The fourth category of plants supported the best growth of *S. schenckii*. A dense hyphal mat developed over the leaf surface, independent from the source of inoculation and independent of any possible nutrient carry over from the inoculum medium source. Abundant spore production was present. Plants providing the best source of nutrients for the growth of *S. schenckii* as a saprophyte included: *Acer kawakamii* Koidz., *Acorus gramineus* Soland., *Adiantum philippense* Linn., *Alpinia intermedia* Gagn., *Alyxia insularis* Kaneh. & Sasak., *Caryopteris mastacanthus* Schau., *Castanopsis carlesii* (Hemsl.) Hay., *Celtis sinensis* Pers., *Clematis tashiroi* Maxim., *Coffea arabica* Linn., *Cotoneaster morrisonensis* Hay., *Cyathula prostrata* (Linn.) Bl., *Daphne odora* Thunb. var. *atrocaulis* Rehd., *Daphniphyllum glaucescens* subsp. *luzonense* (Elm.) Huang, *Euphorbia hirta* Linn., *Fagara nitida* Roxb., *Hedyotis uncinella* Hook. & Arn., *Heteropappus oldhamii* (Hemsl.) Kitam., *Lilium formosanum* Wall., *Lobelia phyramidalis* Wall., *Microtropis japonica* (Fr. & Sav.) Hall. f., *Pueraria tokinensis* Gagn., *Randia cochinchinensis* (Lour.) Merr., *Rhododendron ellipticum* Maxim., *Rosa bracteata* Wendl., *Rubus fraxinifolius* Poir, *Sageretia theezans* (Linn.) Brongn., *Scutellaria rivularis* Wall., *Suzukia shikikunensis* Kudo, *Syzygium densinerrium* Merr. var. *insulare* Chang, *Taiwania cryptomerioides* Hay., *Tarenna kotoensis* (Hay.) Kaneh. & Sasak., *Tricolysia dubia* (Lindl.) Ohwi, *Trichodesma khasianum* Clark., and *Vitex negundo* Linn..

DISCUSSION

The genus *Sporothrix* is a member of the class Deuteromycetes. *Sporothrix schenckii* and *S. beurmanni* are reported as human pathogens (Greer, 1962), however, it is now accepted that *S. schenckii* is the single species and causal agent of sporotrichosis (Hildick-Smith *et al.*, 1964). The name *S. beurmanni* is now recognized as a synonym for *S. schenckii* originally given to isolates that lost their ability to produce the black colony pigment (Emmons *et al.*, 1970). An organism isolated in Taiwan was identified in patients and in nature as *S. schenckii* (Jen, 1963, 1970, Volz *et al.*, 1974). Contact with the organism occurs primarily in outdoor occupations (Moss & McQuown, 1960). Infection in man occurs following traumatic introduction of the organism into tissues or through a break in the skin or mucosa (Hildick-Smith *et al.*, 1964). Entrance into the body can also be gained directly through the lungs by spore inhalation (Buechner, 1971). The organism has permeated the intestinal mucosa by the patient eating contaminated raw fruits or vegetables (Lewis & Hooper, 1948). It also has been shown that protein-deficient diets enhance the susceptibility and the disease severity (Mariat, 1968). Myrvik *et al.* (1974) in a recent study on sporotrichosis considers it probable that infection with *S. schenckii* occurs often in common with many fungal infections. Treatment is infrequent until clinical cases become severe.

Sporothrix schenckii is world wide in distribution, and frequently found in Taiwan soil. It has been isolated previously from soil (Howard and Orr, 1963; Mackinnon *et al.*, 1969). It is the purpose of this study to identify plant species in Taiwan that serve as growth supporting agents for *S. schenckii* in the saprophytic state. Its distribution in the soil and the conditions that enhance its occurrence have to date been the subject of much speculation. Various investigators have traced sporotrichosis epidemics and isolated cases to contact with various vegetation yet no clear explanation is given for the distribution of *S. schenckii* in soil. Temperatures around 26°C and a relative humidity above 92% favor growth of the organism (Mackinnon and Conti-Diaz, 1962; Findley, 1970; Rippon, 1974).

Wooden mine timbers supported heavy growth of the organism in the first large epidemic involving gold mine workers in South Africa (Pijper and Pullinger, 1972; Dangerfield and Gear, 1941; Simson, 1947; Findley, 1970). The fungus has been found growing in nests and burrows of armadillos (Mackinnon *et al.*, 1969), on straw (Silva and Guimaraes, 1964), sphagnum moss (Gastineau *et al.*, 1941; Crevasse and Ellner, 1960; Kedes *et al.*, 1964; D'Alessio *et al.*, 1965),

oak and beach wood litter (Mariat, 1968), grass in basket weaving (Gonzalez Ochoa and Ricoy, 1970; Velasco and Gonzalez Ochoa, 1971), rose thorns (Emmons *et al.*, 1970), barberry thorns (Carter, 1926; Foerster, 1926; Wakefield, 1927; Blair and Yarian, 1928; Weise, 1931), cactus thorns (Lewis and Cuddmore, 1934), beech bark, horsetail plants and oat grains (DeBeurmann and Gougerot, 1908), rough bark of trees (Wilson and Plunkett, 1967), eucalyptus and acacia wood (Mackinnon, 1949), fern plants (DeBeurmann and Gougerot, 1908), wheat (Hopkins and Benham, and Kesten, 1932). Individuals working as florists (Gastineau *et al.*, 1941), farmers, horticulturists (Hildick-Smith *et al.*, 1964) and tree nurserymen (Crevasse and Ellner, 1960; Hays, 1960) have obtained the disease. Other occupations have been mentioned involving contaminated objects (Robinson, 1949), but primarily the source of infection was a plant or plant product.

Animal involvement with sporotrichosis has been reported by previous investigators. Animal bites as the vector for human sporotrichosis or animal lesions from which *S. schenckii* has been isolated were identified in mice (Mackinnon *et al.*, 1964; Emmons *et al.*, 1970; Conant *et al.*, 1971), rats (Meyer, 1915; Anderson and Spector, 1932; Hopkins and Benham, 1932; Mackinnon and Conti-Diaz, 1962), camels (Curasson, 1942), cuttle (Humphreys and Helmer, 1943), horses

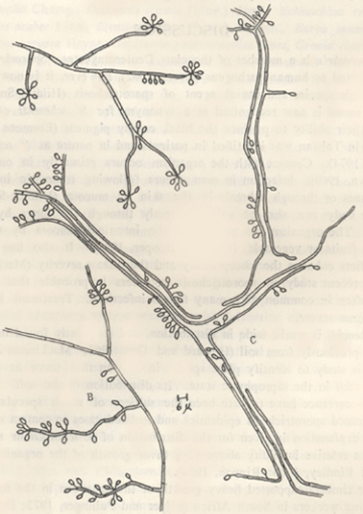

Fig. 1. A. Apical flower arrangement of clustered conidia. B. Conidial formation along the sides of hyphae which increases with colony age. C. Typical parallel and twisted growth of hyphae.

and mules (Meyer, 1915; Ditchfield, 1963; Davis and Worthington, 1964; Kral and Swartzmann, 1964; Fishburn and kelley, 1967; Blood and Henderson, 1968), dogs (Meyer, 1915; Hull, 1955; Kral and Swartzman, 1964; Londero *et al.*, 1964), swine (Smith, 1965), chimpanzees (Saliba *et al.*, 1968), domestic fowl (Meyer, 1915; Saunpers, 1948), cats, hamsters (Jungerman and Schwartzman, 1972), boa constrictors (Gray and Bamber, 1932), flies, fleas, wasps and ants (Hopkins and Benham, 1932; Rippon, 1974). Cold stored meat products have also supported growth of *S. schenckii* (Ahearn and Kaplan, 1969).

The disease in man caused by *S. schenckii* is a chronic involvement of the skin, subcutameous tissue and lymphatic system. Lesions are granulomatous or they may ulcerate and drain. Bones, joints, lungs and the central nervous system are also infection sites with organ systems involved in disseminated infections. Various chemotherapeutic agents and surgery have been selected for disease control (Hildick-Smith *et al.*, 1964; Conant *et al.*, 1971; Rippon, 1974). The organism is a diphasic fungus, producing a yeast colony at 37°C and mycelium with conidia at room temperature. The conidia are oval to pear shaped, 2 to 4 microns by 3 to 5 microns in size, attached singly along the sides of hyphae or terminal on hyphae or in clusters at the end of short hyphal branches (Fig. 1.).

Sporothrix schenckii has been associated with numerous plants, plant products, animals and man. In our study it was learned that abundant hyphal and conidial production on one plant species did not occur in other species of the same genus. Frequently tomentose leaves with thick cuticles were not conducive for fungal growth. Hyphal growth was more dense on the leaf margin or injured areas of the leaf blade. The organism primarily survives as a saprophyte in nature, however, some plant species selected from the Taiwan flora offer good growth support while others do not serve as a nutrient source.

ACKNOWLEDGEMENTS

Special appreciation is extended to Dr. Charles E. DeVol and the National Taiwan University herbarium staff for providing plant specimens for these studies. The assistance and interest of Drs. Zuei-ching Chen and Tseng-chieng Huang is also acknowledged. Support for the mycological studies in Taiwan was received from the U.S.—R.O.C. Cooperative Science Program sponsored by the U.S. National Science Foundation and the National Science Council, R.O.C..

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